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Lacertid Elucidate Both Historical Causes and Morphological Adaptation**



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MATRIX CORRESPONDENCE TESTS ON THE DNA PHYLOGENY OF THE TENERIFE LACERTID ELUCIDATE BOTH HISTORICAL CAUSES AND MORPHOLOGICAL ADAPTATION

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Abstract.—Previous studies using partial regression Mantel tests of matrix correspondence on within-island geographic variation in the color pattern of the Tenerife (Canary Islands) lacertid lizard (*Gallotia galloti*) support natural selection for different north–south climatically determined biotopes but do not support any historical cause. However, tests on the DNA phylogeny based primarily on population data from 57 localities on Tenerife support the hypothesis that there were populations on two putative precursor islands that have come into secondary contact and introgressed after these islands were joined to form Tenerife by the eruption of the Cañadas edifice. Subsequent partial Mantel tests continue to support the hypothesis that color pattern is adapted to the climatic biotopes even when this phylogenetic information is taken into account by (1) testing for color pattern adaptation separately within each lineage and (2) testing for color pattern adaptation across the entire island and simultaneously considering the molecular phylogenetic relationships as representing an alternative explanation. Selection has largely expunged any trace of the geological history from current morphological variation, and the introgression of these island populations after an estimated 0.7 million years of separation gives an insight into the relationships between allopatric divergence and reproductive isolation. [Partial Mantel tests; molecular phylogeny; historical hypotheses; lineage introgression; cytochrome *b* sequence; DGGE; Canary Island lizards; Lacertidae; *Gallotia galloti*.]

The lacertid lizard *Gallotia galloti* exhibits marked geographic variation on the island of Tenerife (Canary Islands) in size (Thorpe and Brown, 1991), shape, scalation (Thorpe and Baez, 1987), and color pattern of sexually mature males (Thorpe and Brown, 1989; Thorpe et al., 1994a). Most of the current island of Tenerife is considered relatively young, although the oldest rocks date back to 15.7 million years ago (Ancochea et al., 1990). There are three areas of ancient basaltic rocks that may represent separate precursor islands: Anaga in the east, Teno in the northwest, and Adeje in the southwest. The two ancient western areas may represent a single precursor with the intervening rocks overlaid by subsequent deposits. The ancient precursor islands are thought to have been joined into a single land mass by a series of eruptions (including the Cañadas and Teide eruptions), which continued until 100,000–200,000 years ago (Ancochea et al., 1990).

The substantial height of Teide (3,718 m) results in altitudinal heterogeneity in climatic conditions, and the high elevation

combined with the predominantly northern trade winds result in a persistent cloud layer on the north-facing slopes at mid and low altitudes (Huetz de Lemps, 1969). There may be a sharp ecotone between the lush, warm, wet north-facing slopes and the barren, hot, arid habitat elsewhere (Thorpe and Brown, 1989). The complexity of the geological history and the pronounced environmental heterogeneity produce several competing hypotheses to explain the geographic variation in lacertids on this island (Thorpe and Brown, 1989; Thorpe et al., 1994a).

The color pattern of sexually mature males, which is the most striking facet of the geographic variation, has been simultaneously tested against a series of competing historical and current ecological hypotheses (Thorpe and Brown, 1989; Thorpe et al., 1994a). The pattern is very closely correlated with different north–south climatic and vegetational biotopes and is thought to be due to an evolutionary balance between sexual selection for bright lateral colors and natural selection for

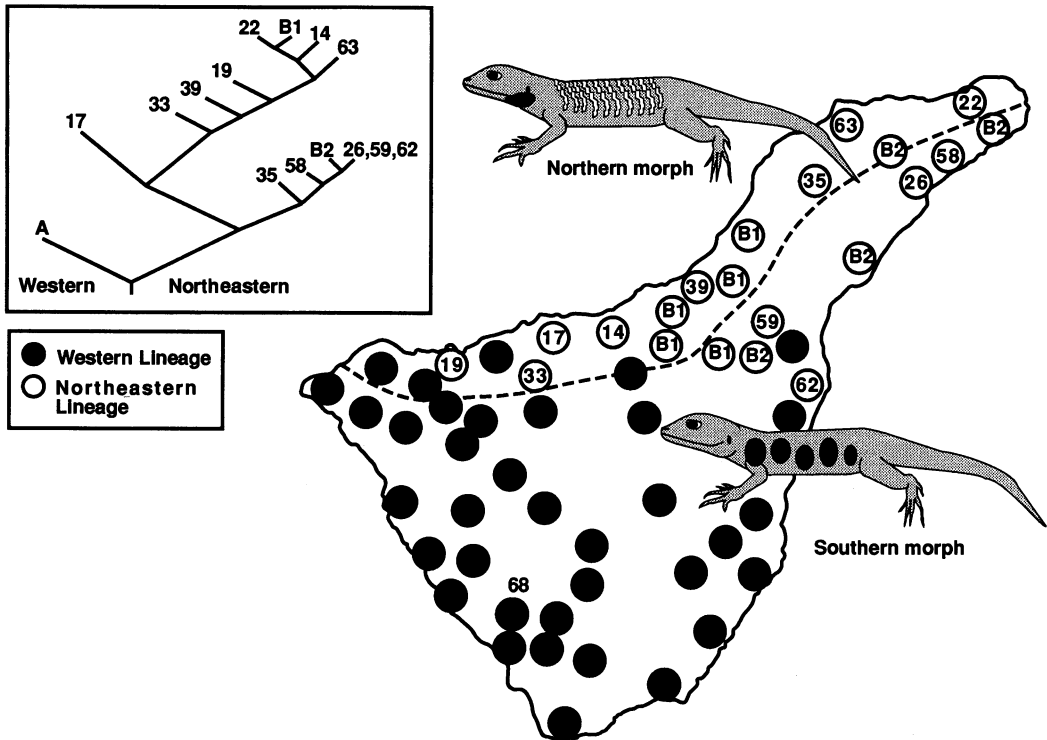


FIGURE 1. An outgroup-rooted Fitch-Margoliash phylogenetic tree, taking into account within-locality variation, shows two main lacertid lizard lineages on Tenerife (western and northeastern); the first with haplotype A and the second with haplotypes B1 and B2 (where locality numbers are given there was more than one haplotype per locality). Irrespective of lineage, populations to the north of the dotted line (produced from contouring mean scores from a canonical analysis of color pattern characters [Thorpe and Brown, 1989]) living in a warm, humid, lush habitat tend to have sexually mature males with dorsal yellow cross bars for disruptive crypsis and lateral blue cheeks sexually selected for intraspecific communication, whereas those to the south of the line, living in a hot, arid, barren habitat, tend to lack disruptive dorsal bars and have lateral blue markings on the trunk.

cryptic dorsal coloration, mediated by different thermal and visual conditions in the two biotopes (Fig. 1). Neither the color pattern nor any of the morphological features support historical hypotheses based on secondary contact after separate evolution on precursor islands (Thorpe and Baez, 1987; Thorpe and Brown, 1989, 1991; Thorpe et al., 1994a).

Matrix correspondence or correlation (Mantel) tests have been used to choose among competing historical evolutionary hypotheses (Sokal et al., 1991; Waddle, 1994), but the type of data can prejudice the conclusions because morphology can be subject to strong selective forces (Price et al., 1984; Brown et al., 1991; Malhotra

and Thorpe, 1991) that can compromise the historical record. Consequently, in this paper hypotheses of the phylogenetic relationships are based on molecular data. Alternative historical explanations were tested for the molecular phylogeny, and the relationship between biotope and color pattern was retested using partial Mantel tests in light of this molecular phylogenetic information.

MOLECULAR PHYLOGENY

A comprehensive set of 67 localities were sampled, representing all geological and ecological regions of Tenerife for the previous morphological studies of *G. galloti* (Thorpe and Brown, 1989, 1991). The sam-

ples for molecular analyses were based on the same series of localities used by Thorpe and Brown (1991) with the addition of locality 68 (Fig. 1) and the exclusion of several localities depending on the type of molecular data under consideration (Appendix 1).

Total DNA was extracted from autotomized tail tips stored in 70% ethanol using either standard phenol or Chelex (BioRad) procedures. A 316-base-pair section (excluding primers) of the mitochondrial cytochrome *b* gene was amplified by polymerase chain reaction (PCR) using variants of primers L14841 and H15149 (Kocher et al., 1989). Denaturing gradient gel electrophoresis (DGGE) (Abrams and Stanton, 1992) on a G+C-rich PCR product was used to test sequence identity (Sheffield et al., 1989; Myers, 1993). This procedure revealed three main haplotypes: A, B1, and B2. Haplotype A tends to be found in the west, and haplotypes B1 and B2 tend to be found in the northeast (Fig. 1).

Direct double-stranded sequencing (Winship, 1989) resulted in 238 consistently sequenceable bases (Appendix 2, GenBank U51299–U51301). A Fitch–Margoliash phylogenetic tree was produced using PHYLIP from a matrix representing distances among localities, which takes into account within-locality variation across these 238 bases from 57 sample localities (Felsenstein, 1993) (Fig. 1; Appendix 1). Where more than one haplotype was found at a locality, the mean pairwise distance between localities was used across all individuals in both localities. The tree was rooted with *Psammodromus* and congeners *Gallotia atlantica* and *G. stehlini* (Thorpe et al., 1994b). There are two main lineages (western and northeastern), the first with haplotype A and the second with haplotypes B1 and B2. (Fig. 1). In addition, a Wagner tree from PAUP (Swofford, 1990) and a Fitch–Margoliash tree without a molecular clock from PHYLIP (Felsenstein, 1993) were constructed using modal haplotypes and the same outgroups as above (Appendix 1).

TESTING THE MOLECULAR PHYLOGENY AGAINST ALTERNATIVE HISTORICAL HYPOTHESES

Observed patterns of variation in morphological and molecular data can be tested with Mantel tests against patterns generated from putative causes. In a simple pairwise test, a parameter such as a correlation or regression coefficient is taken as measure of the association between two matrices, one of which represents the pattern of observed variation (dependent variable) and the other represents the pattern generated by the putative cause (independent variable). Repeated randomization of the localities (rows and columns) in one of the matrices (e.g., dependent matrix) and recomputation of the statistic measuring association gives a distribution for the statistic that allows testing of the null hypothesis of no association.

When there is more than one putative cause, they may be tested separately by pairwise tests or taken as a series of independent variables and tested simultaneously by partial correlation (Smouse et al., 1986) or partial regression (Manly, 1991; Thorpe and Baez, 1993). Partial regressions or partial correlations are particularly useful when, as is often the case, different hypotheses generate intercorrelated patterns. Here we used standardized regression coefficients between pairs of matrices and standardized absolute partial regression coefficients (with a standard nonstepwise procedure) when considering a series of independent variable matrices simultaneously (Manly, 1991; Thorpe and Baez, 1993).

Several putative causes may be additive in their effect; for example, morphological variation may be influenced by both historical events and natural selection for current ecological conditions. Under these circumstances, a test of multiple hypotheses using partial regression (or correlation) may indicate that more than one of these hypotheses cannot be rejected. However, some putative causes may be logically mutually exclusive. If partial regression (or correlation) does not allow rejection of two

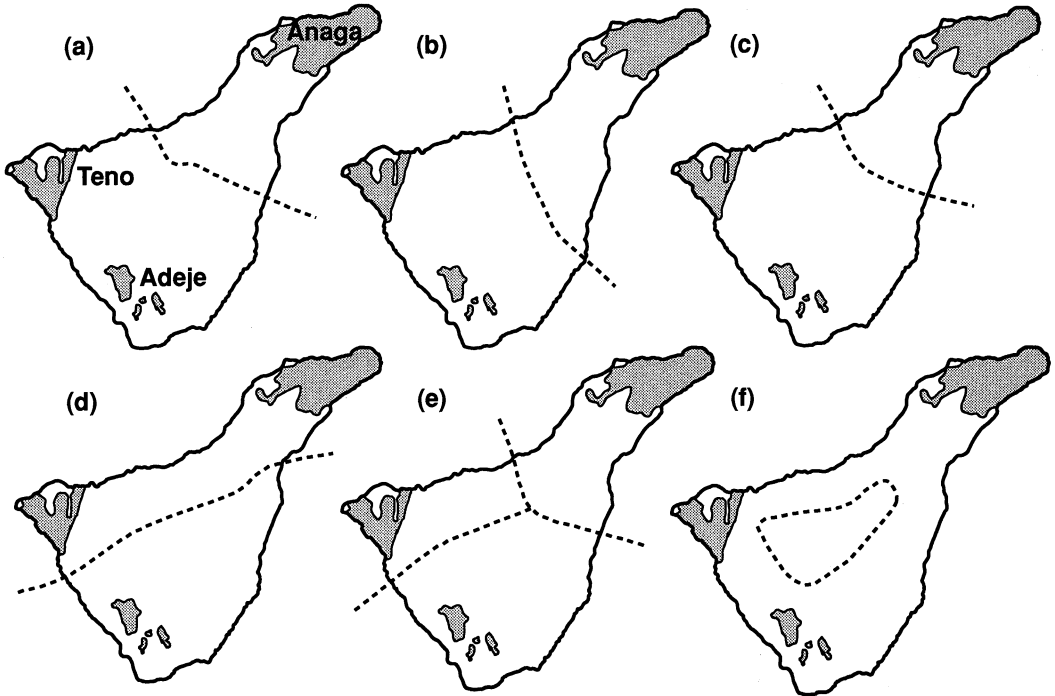


FIGURE 2. Patterns generated by hypotheses A–F (a–f, respectively). Shaded areas representing ancient rocks on Tenerife in areas of Teno (northwest), Adeje (southwest), and Anaga (northeast).

or more mutually exclusive causes, then it is self-evident that there is insufficient evidence to choose among the unrejected hypotheses.

The molecular phylogeny of this comprehensive set of lizard populations from across Tenerife indicates northeastern and western lineages (Fig. 1). In this study, a molecular phylogeny is represented in a Mantel test as a matrix of patristic distances among populations. Independent series of Mantel tests were run for each of the three molecular phylogenies with the patristic distances as the dependent matrix.

A series of seven hypotheses were erected to explain this phylogenetic pattern: five from geological vicariance, one from cloud-induced vicariance, and one from geographic proximity. These hypotheses were first tested against the molecular patristic distances one at a time using simple pairwise Mantel tests, and those not rejected were then simultaneously tested using partial regressions Mantel tests. These

hypotheses can also be represented by dissimilarity matrices among localities, and patterns were generated from the hypotheses.

- A. Anaga and Adeje. This hypothesis assumes Anaga and Adeje had differentiated populations that met secondarily once these precursor islands joined after the eruption of Cañadas, with equal rates of spread measured from the outer edges of the precursors (Fig. 2a). The dissimilarity matrix representing this hypothesis has elements of 1 and zero representing the dissimilarity between localities on the opposite side of the line and on same side of the line, respectively (where the line represents the contact between hypothesized ranges).
- B. Anaga and Teno. As for hypothesis A except Teno and Anaga are considered the two precursor islands with populations (Fig. 2b).

TABLE 1. Mantel test probabilities for DNA phylogenies against alternative historical hypotheses. The null hypothesis of no association is rejected if the tabulated value of P is less than the critical value of 0.0015 ($P < 0.05$ divided by 33 tabulated values to give the Bonferroni correction). Asterisks indicate significant hypotheses. Probabilities are derived from 10,000 randomizations. Three molecular lizard phylogenies are taken in turn: a Fitch–Margoliash tree on a genetic distance taking into account within-locality variation (FM within), a Fitch–Margoliash tree on modal haplotypes at each locality (FM modal), and a Wagner tree on modal haplotypes at each locality (Wagner modal). In analysis 1, simple pairwise Mantel tests were conducted between DNA phylogenies and the seven hypotheses (A–G) given in the text. In analysis 2, the four significant hypotheses from analysis 1 were tested simultaneously against DNA phylogenies using partial regression Mantel tests.

Analysis	Dependent matrix	Hypotheses						
		A	B	C	D	E	F	G
1	FM (within var)	0.0001*	0.0001*	0.0001*	0.7408	0.0750	0.3308	0.0004*
	FM (modal)	0.0001*	0.0001*	0.0001*	0.0367	0.4805	0.6810	0.0001*
	Wagner (modal)	0.0001*	0.0001*	0.0001*	0.0182	0.7066	0.6854	0.0002*
2	FM (within var)	0.1275	0.1894	0.0001*				0.7229
	FM (modal)	0.4498	0.4368	0.0001*				0.7881
	Wagner (modal)	0.4611	0.4413	0.0001*				0.7889

- C. Anaga and Teno + Adeje. As for hypothesis A, except Teno and Adeje are considered to have been a single precursor island (Fig. 2c).
- D. Teno and Adeje. This hypothesis assumes that Teno and Adeje were (the only) separate precursor islands with populations (Fig. 2d).
- E. Anaga, Teno, and Adeje. This hypothesis assumes all three ancient areas were separate precursor islands with populations (Fig. 2e).
- F. Cloud ring. This hypothesis assumes that there is a ring of cloud around Tenerife which separates low altitude and high altitude populations (Fig. 2f) sufficiently for them to have separate evolutionary histories (Pasteur and Salvadio, 1985).
- G. Geographic proximity. Closer populations have a greater opportunity for gene flow. Moreover, there may be a factor with a geographic component that has not been explicitly represented by one of the above hypotheses. This hypothesis is represented by a matrix of geographic distances among localities.

Hypotheses A, B, and C are all similar in that they hypothesize a single eastern and a single western precursor population. They differ in the extent of the distribution of the western precursor population, with

A representing its restriction to Adeje (southwest), B representing its restriction to Teno (northwest), and C representing its distribution as a single entity across both of these ancient regions. These hypotheses are very similar and consequently generate very similar patterns.

When the phylogenies were each tested against each of these seven hypotheses in turn, using simple pairwise Mantel tests, then hypotheses D, E, and F were rejected (Table 1: analysis 1), but geographic proximity (G) and hypotheses proposing northeastern and western precursor islands (A, B, C) were not rejected. The hypotheses that could not be rejected by a simple pairwise Mantel test were then tested simultaneously by a partial regression Mantel test that takes into account the intercorrelation among patterns generated by the alternative hypotheses. Thus, for each of the three molecular phylogenies, only hypothesis C could not be rejected (Table 1: analysis 2). Consequently, these tests suggest that the western ancient rocks of Adeje and Teno had a precursor population that has expanded to meet that from Anaga secondarily after these ancient islands were joined to form Tenerife by the volcanism between them.

The estimated time of divergence between the western and northeastern haplotypes is ca. 0.7 million years, based on

2.5%/million years (Kocher et al., 1989). Anaga was separate from the western precursors (Teno and Adeje) at that time and may only have been joined by suitable habitat as recently as 0.1–0.2 million years ago or later (Ancochea et al., 1990). Consequently, the time of DNA divergence is compatible with the timing of the geological events on the island that are pertinent to hypothesis C.

USING THE PHYLOGENY TO RETEST ECOGENETIC ADAPTATION

Independent contrasts (Felsenstein, 1985) may be used to correlate morphological and environmental variables taking into account phylogenetic relationships, but at the intraspecific level Mantel tests have the advantage that they are suitable for geographic populations that may not be phylogenetically distinct. Moreover, Mantel tests are more appropriate for considering the essentially matrix form of geographic distance among populations and multivariate generalized data sets encountered in this and other intraspecific studies (Thorpe et al., 1995; Thorpe, 1996).

The DNA phylogeny (a Fitch–Margoliash tree taking into account within-locality variation in sequence) in this intraspecific study was used in a series of partial Mantel tests to retest the cause of the generalized color pattern variation in lacertids. The data for geographic variation are based on the Mahalanobis distance among localities generalized across six characters recorded from at least five sexually mature males from each of the same 56 localities used in both this molecular phylogeny (Appendix 1) and the original study of color pattern (Thorpe and Brown, 1989; Thorpe et al., 1994a).

The molecular phylogenetic relationships were taken into account in two ways when retesting the relationship between color pattern and climatic biotope. First, the populations in the two lineages (western and northeastern, Fig. 1) were tested separately for the association between color pattern (dependent variable) and two putative causes, climatic biotope and geographic proximity (two independent vari-

ables), using partial regression Mantel tests. In both the western lineage (Table 2: analysis 1) and the northeastern lineage (Table 2: analysis 2), the association between male color pattern and climatic biotope could not be rejected. Second, the molecular phylogenetic relationships were allowed for by including the patristic distance matrix as one of the three dependent variables in a partial regression Mantel test, where the color pattern is the dependent variable tested against three dependent variables (molecular phylogeny patristic distances, climatic biotopes, geographic proximity). This analysis indicated a significant association between male color pattern and climatic biotopes (Table 2: analysis 3), even when the phylogenetic relationships (and proximity) were partialled out.

Anal- ysis	Dependent matrix	Hypotheses		
		DNA phylogeny	Proximity	N-S biotopes
1	Color pattern in western lineage		0.0023*	0.0024*
2	Color pattern in northeast lineage		0.4258	0.0002*
3	Color pattern (all island)	0.0343	0.0190	0.0001*

DISCUSSION

This study shows how partial Mantel tests can be used to choose among alternative historical explanations for molecu-

lar phylogenetic relationships. These tests support the hypothesis that the different molecular lineages of lacertids on Tenerife are a consequence of secondary expansion of predifferentiated populations on a pair of precursor islands (one in the east, the other in the west) after these precursors were joined by volcanic eruption between them. Although this result (i.e., a north-eastern precursor in Anaga and some sort of western precursor) appears robust, one should exercise caution in deducing the exact location of the western precursor. The set of tests employed in this study indicate that the most likely explanation is a single western precursor that occupied both Adeje and Teno. However, the other hypotheses that assumed a single western precursor (restricted to either Adeje or Teno) generated very similar patterns. Either of these may have been selected as the most likely hypothesis if different assumptions had been adopted (i.e., assumptions other than an even rate of population spread from the outer edges of the ancient precursor areas).

This study also illustrates how molecular phylogenetic information can be used when Mantel tests are employed to test for ecogenetic adaptation in other features. In this example, two methods were employed to retest the hypothesis that the color pattern of sexually mature *Gallotia galloti* males is related to the climatic and vegetational biotopes on Tenerife. First, Mantel tests were used with hypotheses separately within each eastern and western lineage. Second, the molecular phylogeny was used as one of the independent variables in a partial Mantel test (Thorpe et al., 1995; Thorpe, 1996) when the variation in male color pattern was being tested against potentially explanatory (independent) variables such as habitat type. This approach uses partial regression techniques to simultaneously consider the past phylogeny and current habitat type (and other factors) against the observed pattern of geographic variation in color pattern. Both these testing procedures support habitat type as the explanation for north-south

variation in color pattern of sexually mature male lizards.

Selection appears to have largely expunged any influence of the geological history from the aspects of the color pattern variation under consideration. Moreover, there is complete introgression (reticulation of the lineages) of these island populations after having diverged on separate islands ca. 0.7 million years ago. This unusual situation gives some insight into the relationship between the extent of divergence in allopatry and retention of the capacity to completely introgress.

The western lineage, unlike the north-eastern lineage, lacks molecular genetic diversity. This finding could be the result of inadequate information, and sequence from a larger section of the mitochondrial genome might reveal some diversity. In contrast, this finding could indicate a severe population bottleneck in the past, perhaps caused by a dramatic decrease in population range and size associated with the volcanic eruptions that joined the western and eastern precursor islands.

Although phylogeographic studies using morphology are possible, e.g., the post-Pleistocene range expansion of the *Natrix natrix* complex can be deduced from morphology (Thorpe, 1984), it is only with the advent of molecular methods that this area has become popular. Molecular phylogeographic studies cover a wide range of organisms from butterflies (Sperling and Harrison, 1994) to humans (Bowcock et al., 1994) (reviewed by Avise, 1994), but they usually involve a much larger spatial scale than that of the present study and do not usually involve explicit probabilistic tests of alternative historical hypotheses. Nor do these studies use the molecular phylogeographic information to assist in testing the geographic variation in morphology against ecogenetic hypotheses. Hence, apart from a brief preliminary report (Thorpe et al., 1995), this paper represents one of the first attempts to investigate microphylogeographic relationships on one island to test a molecular phylogeny against alternative historical hypotheses and to use a molecular phylog-

ery to help test causal hypotheses for geographic variation in morphology.

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REFERENCES

- ABRAMS, E. S., AND V. P. STANTON, JR. 1992. Use of denaturing gradient gel electrophoresis to study conformational transitions in nucleic acids. *Methods Enzymol.* 212:71-104.
- ANCOCHEA, E., J. M. FUSTER, E. IBARROLA, A. CENDREIRO, J. COELLO, F. HERNAN, J. M. CANRAGREL, AND C. JAMOND. 1990. The volcanic evolution of the island of Tenerife (Canary Islands) in the light of new K-Ar data. *J. Volcan. Geotherm. Res.* 44:231-249.
- AVISE, J. A. 1994. *Molecular markers, natural history and evolution.* Chapman Hall, New York.
- BOWCOCK, A. M., A. RUIZLINARES, J. TOMFOHRDE, E. MINCH, J. R. KIDD, AND L. L. CAVALLI-SFORZA. 1994. High resolution of human evolutionary trees with polymorphic microsatellites. *Nature* 368:455-457.
- BROWN, R. P., R. S. THORPE, AND M. BAEZ. 1991. Lizards on neighbouring islands show parallel within-island micro-evolution. *Nature* 352:60-62.
- FELSENSTEIN, J. 1985. Phylogenies and the comparative method. *Am. Nat.* 125:1-15.
- FELSENSTEIN, J. 1993. *PHYLIP manual*, version 35p. Department of Genetics, Univ. Washington, Seattle.
- HUETZ DE LEMPS, A. 1969. *Le climat des Iles Canaries.* Publ. Facul. Lett. Sci. Hum. 54. Sorbonne, Paris.
- KOCHER, T. D., W. K. THOMAS, A. MEYER, S. V. EDWARDS, S. PÄÄBO, F. X. VILLABLANCA, AND A. C. WILSON. 1989. Dynamics of mitochondrial DNA evolution in animals: Amplification and sequencing with conserved primers. *Proc. Natl. Acad. Sci. USA* 86:6196-6200.
- MALHOTRA, A., AND R. S. THORPE. 1991. Experimental detection of rapid evolutionary response in natural lizard populations. *Nature* 353:347-348.
- MANLY, B. F. J. 1991. *Randomization and Monte Carlo methods in biology.* Chapman Hall, London.
- MYERS, R. M. 1993. A simple and efficient method for attachment of a 40-base pair GC-rich sequence to PCR amplified DNA. *BioTechniques* 12:386-387.
- PASTEUR, G., AND S. SALVIDIO. 1985. Notes on the ecological genetics of *Gallotia galloti* populations from Tenerife. *Bonn. Zool. Beitr.* 36:553-556.
- PRICE, T., P. GRANT, H. GIBBS, AND P. T. BOAG. 1984. Recurrent patterns of natural selection in a population of Darwin's finches. *Nature* 309:787-789.
- SHEFFIELD, V. C., D. R. COX, L. S. LERMAN, AND R. M. MYERS. 1989. Attachment of a 40-base-pair G+C-rich sequence (GC-clamp) to genomic DNA fragments by the polymerase chain reaction results in improved detection of single-base changes. *Proc. Natl. Acad. Sci. USA* 86:232-236.
- SMOUSE, P. E., J. LONG, AND R. R. SOKAL. 1986. Multiple regressions and correlation extensions of the Mantel test of matrix correspondence. *Syst. Zool.* 35: 627-632.
- SOKAL, R. R., N. L. ODEN, AND A. C. WILSON. 1991. Genetic evidence for the spread of agriculture in Europe by demic diffusion. *Nature* 351:143-145.
- SPERLING, F. A. H., AND R. G. HARRISON. 1994. Mitochondrial DNA variation within and between species of the *Papilio machaon* group of swallowtail butterflies. *Evolution* 48:408-422.
- SWOFFORD, D. L. 1990. *PAUP: Phylogenetic analysis using parsimony*, version 3.0r. Illinois Natural History Survey, Champaign.
- THORPE, R. S. 1984. Primary and secondary transition zones in speciation and population differentiation: A phylogenetic analysis of range expansion. *Evolution* 38:233-243.
- THORPE, R. S. 1996. The use of DNA divergence to help determine the correlates of evolution of morphological characters. *Evolution* 50:524-531.
- THORPE, R. S., AND M. BAEZ. 1987. Geographic variation within an island: Univariate and multivariate contouring of scalation, size and shape of the lizard *Gallotia galloti*. *Evolution* 41:256-268.
- THORPE, R. S., AND M. BAEZ. 1993. Geographic variation in the scalation of the lizard *Gallotia stehlini* within the island of Gran Canaria. *Biol. J. Linn. Soc.* 48:75-87.
- THORPE, R. S., AND R. P. BROWN. 1989. Microgeographic variation in the colour pattern of the lizard *Gallotia galloti* within the island of Tenerife: Distribution, pattern and hypothesis testing. *Biol. J. Linn. Soc.* 38:303-322.
- THORPE, R. S., AND R. P. BROWN. 1991. Microgeographic clines in the size of mature male *Gallotia galloti* (Squamata: Lacertidae). *Herpetologica* 47:28-37.
- THORPE, R. S., R. P. BROWN, M. DAY, A. MALHOTRA, D. P. MCGREGOR, AND W. WÜSTER. 1994a. Testing ecological and phylogenetic hypotheses in micro-evolutionary studies. Pages 189-206 in *Phylogenetics and ecology* (P. Eggleton and R. Vane-Wright, eds.). Academic Press, London.
- THORPE, R. S., A. MALHOTRA, H. BLACK, J. DALTRY, AND W. WÜSTER. 1995. Relating geographic pattern to phylogenetic process. *Philos. Trans. R. Soc. B* 349: 61-68.
- THORPE, R. S., D. P. MCGREGOR, A. M. CUMMING, AND W. C. JORDAN. 1994b. DNA evolution and colonization sequence of island lizards in relation to geological history: mtDNA RFLP, cytochrome *b*, cytochrome oxidase, 12S rRNA sequence, and RAPD nuclear analysis. *Evolution* 48:230-240.
- WADDLE, D. M. 1994. Matrix correlation tests support a single origin for modern humans. *Nature* 368:452-454.
- WINSHIP, P. R. 1989. An improved method for directly sequencing PCR amplified material using dimethyl sulfoxide. *Nucleic Acids Res.* 17:1266.

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APPENDIX 1

Localities used for the Fitch–Margoliash tree, taking into account within-locality variation. Locality numbers follow those of Thorpe and Brown (1991) with the addition of locality 68 (Fig. 1); sample sizes are in parentheses: 1 (5), 2 (3), 3 (5), 4 (2), 5 (3), 8 (4), 9 (2), 10 (1), 12 (1), 13 (6), 14 (2), 15 (5), 16 (5), 17 (3), 18 (1), 19 (4), 20 (4), 21 (5), 22 (11), 23 (1), 25 (3), 26 (4), 27

(1), 28 (4), 29 (4), 30 (2), 31 (3), 32 (3), 33 (5), 35 (4), 36 (2), 38 (1), 39 (2), 40 (2), 42 (1), 43 (1), 45 (4), 46 (4), 47 (1), 48 (2), 49 (5), 50 (4), 51 (4), 52 (4), 53 (2), 54 (4), 55 (1), 58 (4), 59 (4), 60 (1), 61 (1), 62 (4), 63 (4), 64 (1), 65 (1), 67 (1), 68 (4).

The localities used for the Wagner tree and Fitch–Margoliash tree using modal haplotypes are as above, except localities 14, 19, 39, 58, and 63 are excluded because they have no clear modal haplotype.

APPENDIX 2

Sequence of *Gallotia galloti* haplotypes A, B1, and B2 from Tenerife aligned against *G. atlantica* (Thorpe et al., 1994b). Numbers at the beginning and end of the sequence correspond to those of the human mitochondrial genome, and the bold letters above the middle of each codon refer to the translated amino acid.

	G L C L I I Q I I T G L F L A M H Y N A D
A	¹⁴⁸⁵⁸ GGACTTTGCCTNATTATCCANATCATTACAGGCCTATTCTTGGCAATACACTACAACGCAGA
B1A.....T.A.....
B2T.A.....A.....
<i>atlantica</i>A.....T.A.....A.....G.....

	I N S A F S S I A H I H R D V Q H G W L
A	CATCAACTCCGCATTCTCATCCATCGCCACATTCATCGTGACGTCCAACACGGTTGATTGA
B1T.....
B2T.....
<i>atlantica</i>	..T.....T.....C.C.....T.T.....T.....C.T.

	I R N I H A N G A S L F F I C I Y M H I G
A	TCCGAAATATCCACGCCAATGGAGCCTCACTATTTTTTATCTGCATCTACATACACATTGGA
B1
B2
<i>atlantica</i>C.....G.....

	R G L Y Y G S Y L F T E T W N I G V
A	CGAGGCCTATACTACGGCTCATACTTATTTACTGAAACCTGAAACATCGGAGTAC ¹⁵⁰⁹⁸
B1C.....T.....
B2N.....
<i>atlantica</i>	..T.....G.....GT.....T.....